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The complexation of metallothionein with cadmium ion and its conformational study [α,β]

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Abstract

The circular dichroic (CD) spectra of native mouse liver MT-2 (pH 6.10) and re-constituted MT (pH adjusted from 6.10 to 1.84, and then raised to 10.70) have been measured to determine if there are any conformational differences between them. The pH value corresponding to the complete dissociation of Cd^{2+} from rabbit MT-1 is 2.8. Compared with the data derived from a cadmium-selective electrode, the CD spectra of native rabbit liver MT-1 at different pH values (8.19, 4.44, 3.61, 2.94 and 1.92) demonstrate that the release of the metal ions with accompanied by a conformational change in the MT and that the metal–thiolate bond is important in maintaining the secondary structure of MT. At pH 8.19 the addition of excess Cd^{2+} results in a significant change in the CD spectrum of rabbit MT-1, indicating that some metal clusters have been broken.

INTRODUCTION

Metallothionein (MT) is a low molecular weight, sulphydryl-containing protein that binds strongly with a number of metal ions, including those of zinc, cadmium, copper, mercury, etc. It has been isolated from a wide variety of mammalian species, as well as from plants and microorganisms [1–3]. All the sulphydryl groups of the cysteine residues are considered to be involved in binding the metal ions with an approximately tetrahedral arrangement of sulphur atoms around each metal ion [4]. Circular dichroism has proved to be a powerful method for studying the conformation of MTs [5–12]. In this paper, potentiometric techniques using a cadmium-ion-selective electrode and circular dichroic (CD) measurement were employed to study the metal coordination and conformation of mouse MT-2 and rabbit MT-1.

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EXPERIMENTAL

MT was purified as Cd,Zn–protein from mouse and rabbit, induced with $CdCl_2$. The concentration of MT was determined by absorption at 254 nm. The metal content of MT was determined by flame atomic absorption spectroscopy (Beijing Geologic Instrument factory of China, GGX-1). The data are 1.9 mol Cd per mole of protein for mouse MT-2, and 3.1 mol Cd and 0.6 mol Zn per mole of protein for rabbit MT-1. CD measurements were carried out with a Jasco J-500 automatic recording spectropolarimeter, calibrated with D-10 camphorsulphonic acid, in a quartz cell with a path-length of 1.0 cm. Each CD spectrum reported was the average of four scans. The ellipticity (θ) was calculated assuming a molecular weight of 6700, and a mean residue weight of 110 was taken for evaluating θ below 220 nm. Water was distilled twice. The pH of a solution was adjusted adding 3 M HCl or 4 M NaOH using a micro-injector with stirring, and was measured on an Orion 811 pH/mV meter with an accuracy of 0.02. A solution of 0.428 M $CdCl_2$ was used in adding excess Cd^{2+}. The concentration of Cd^{2+} in rabbit MT-1 solution was determined with an Orion 94-48 Cd-ion-selective electrode.

RESULTS AND DISCUSSION

CD spectra above 220 nm

The CD spectra of native mouse MT-2 (pH 6.10), metal-free protein, namely apoMT (pH adjusted to 1.84) and metal reconstituted MT (pH then raised to 10.70) were measured separately. The bands at 260, 238 and 224 nm are characteristic of Cd,Zn–MT where the Cd/Zn ratio is reasonably high, and the spectrum is very similar to the CD spectrum of Cd–MT [5] (Fig. 1).

Because aromatic acids and disulphide bridges are absent in MT, these bands must be attributed to the metal–thiolate chromophore. Lowering the pH to 1.84, the Cotton effects above 220 nm disappear and a shoulder appears at 224 nm. This is due to the dissociation of metal–thiolate chromophore as all the cysteins are protonated at that pH.

The X-ray diffraction crystallographic analysis of native rat liver (Cd_5, Zn_2)–MT-2 [13] shows that there are two metal clusters in the MT: one contains four metal ions (α domain) and the other three (β domain). The CD spectrum of cadmium-containing MT is sensitive to the bridging pattern at the binding sites, and it may be used to demonstrate the presence of the metal clusters.

When the pH was changed from 1.84 to 10.70, the metal ions bonded with the MT again and the CD spectrum of the reconstituted MT was identical

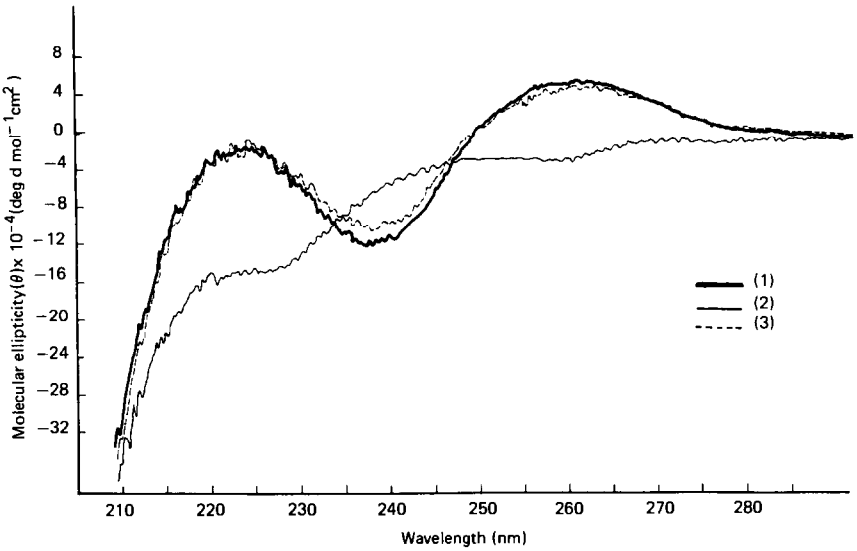

Fig. 1. pH dependency of the circular dichroic spectrum of mouse MT-2, protein concentration 1.04×10^{-5} M: (1) pH 6.10 (native); (2) pH 1.84 (apo); (3) pH 10.70 (reconstituted).

with that of native mouse MT-2 (pH 6.10). This reveals that they have almost the same structure. The CD spectrum at pH 10.70 was recorded 12 hours later, after the pH had been raised from 1.84. The reconstitution of the MT does not seem to be instantaneous: a time effect has to be considered.

The effect of adding excess $CdCl_2$ on the CD spectrum of native MT-1 is shown in Fig. 2. The band at 261 nm exhibits a blue shift with a loss in intensity when 4 equiv. Cd^{2+} is added, and then its intensity increases when 10 and 60 equiv. Cd^{2+} are added. The band at 225 nm decreases continuously, but the band at 240 nm did not change substantially. Native MT can coordinate with 7 equiv. Cd^{2+} or Zn^{2+}, but in the presence of excess Cd^{2+} the considerable changes in the CD spectrum show that MT binds with more than 7 equiv. Cd^{2+} and that the conformation of MT is changed. This behaviour of rabbit MT-1 (pH 8.19) is very similar to rat MT-2 at pH 7 [6,10], and suggests that the coordination mode is the same for the two MTs from different sources. Law and coworkers indicate that the α domain remains as Cd_4 while the β domain can bind up to six mol-equivalents of Cd^{2+} [10].

The effect of pH on the CD spectrum of native rabbit MT-1 is shown in Fig. 3. The bands above 220 nm are diminished at lower pH and a shoulder appears at 224 nm below pH 1.92, as with mouse MT-2, Fig. 1. Figure 4 shows that when the pH reached 2.8 the concentration of Cd^{2+} in the MT solution remained constant, i.e. Cd^{2+} dissociated completely from MT at this pH. During the dissociation, the band at 261 nm shows a slight blue

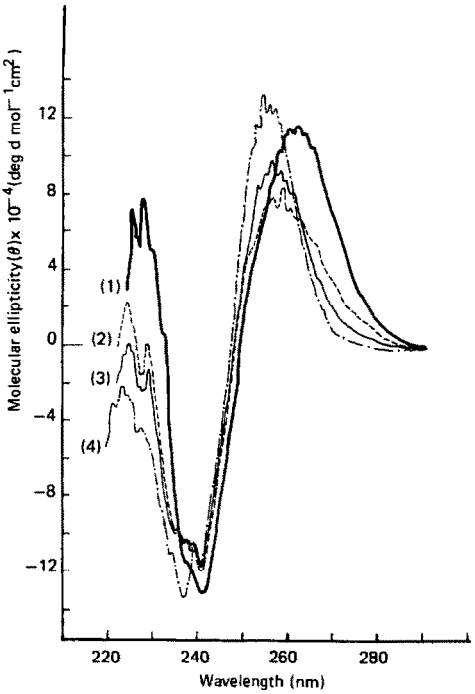

Fig. 2. The effect of adding excess Cd^{2+} to a sample of rabbit MT-1 on its circular dichroic spectrum, protein concentration 2.1×10^{-5} M: (1) native; (2) 4; (3) 10; and (4) 60 mol. equivalents Cd^{2+}.

shift and splits into two small peaks at pH 2.94. Because the CD spectra exhibit changes in shape as well as in amplitude, we infer that the release of metal ions from native rabbit MT-1 may be accompanied by structural changes in the binding sites.

CD spectra below 220 nm

Circular dichroism in the far-ultraviolet region has proved useful in the study of the secondary structure of proteins. According to observations on modal polypeptide, random coils correspond to the negative CD band near 200 nm, while β turns will result in a negative Cotton effect in the 221–225 nm region and a positive band in the 198–205 nm region [14]. An earlier study of circular dichroism showed that chicken Cd,Zn–MT exhibited a negative Cotton effect at 200 nm, which was unaffected by dissociation of the metal ions [5,7]. Thus, the secondary structure of MT was regarded as random coils, not α-helix and β-pleated sheets. However, Raman and IR studies in 1986 suggested that metal re-constituted rabbit MT-1 consisted largely of β turns of type II; in contrast, the metal-free (apo) protein

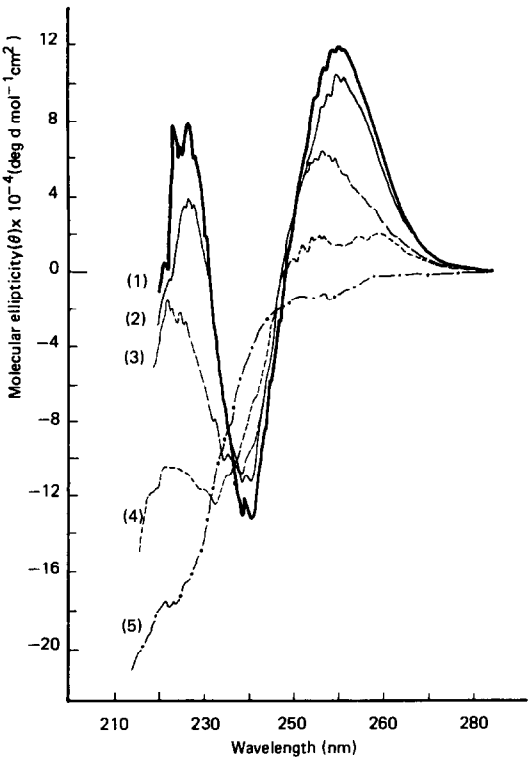

Fig. 3. pH dependency of the circular dichroic spectrum of rabbit MT-1, protein concentration 2.1×10^{-5} M: (1) pH 8.19 (native); (2) pH 4.44; (3) pH 3.61; (4) pH 2.94; (5) pH 1.92.

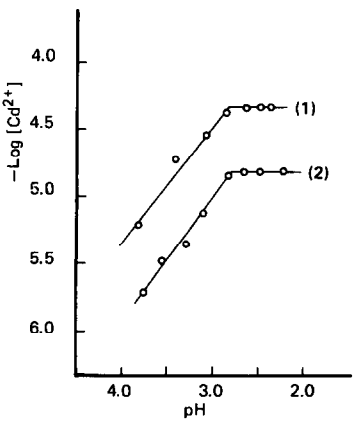

Fig. 4. Log $[Cd^{2+}]$ vs. pH curves for rabbit MT-2: (1) $C = 0.96\times10^{-5}$ M; (2) $C = 0.52\times10^{-5}$ M.

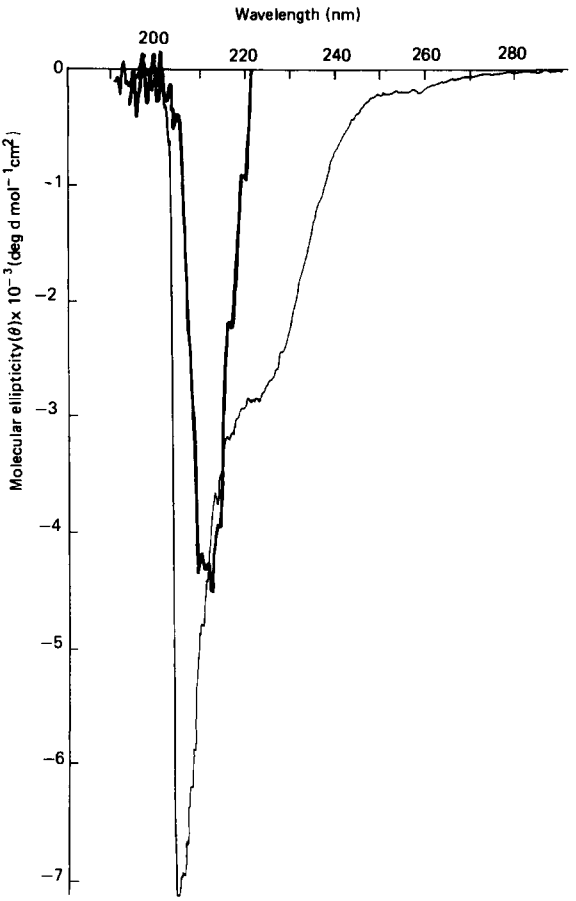

Fig. 5. The effect of dissociation of metal ions on the circular dichroic spectrum of rabbit MT-1 in the far-ultraviolet region, protein concentration 2.1×10^{-5} M: (1) native MT, pH 8.19; (2) apoMT, pH 1.92.

displayed a predominantly disordered conformation; but their CD spectra near 200 nm did not show much difference [11].

In Fig. 5, the native rabbit MT-1 at pH 8.19 displays a negative CD band at 211 nm, but the metal-free MT (pH 1.92) has a much stronger negative band at 205 nm. This change in the CD spectrum demonstrates that a large number of the β turns in native rabbit MT-1 become random coils in apoMT. The dissociation of metal ions seems to damage the β turns, thus changing the protein conformation. But for the chicken Cd,Zn–MT [5,8] the Cotton effect near 200 nm was unaffected by the dissociation of metal ions, so it seems that the metal–thiolate bond plays a much more important role in maintaining the secondary structure of native rabbit MT-1.

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